

**Supplementary Table S1: Plasmids and Oligonucleotides****3A: Plasmids**

pHD no.	description	primers
2101	CNOT10 stem loop TB927.10.8720 Hygro: phd1145 cut HindIII/BamHI + ligate CNOT10 RNAi stem loop HindIII/BgIII	cz4688, 4689
1846	N-terminal V5 in situ NOT1	Schwede et al. 2008
2211	N-terminal V5 in situ CAF1 TB927.6.600: 5'UTR NotI/XbaI, ORF XbaI/ApaI	cz3919, 3920, 3921, 3922
2212	N-terminal V5 in situ CNOT10 TB927.10.8720: 5'UTR NotI/XbaI, ORF XbaI/ApaI	cz4725,4726,4715,4716
2309	in situ N-terminal TAP-tagged CNOT10: p2676 cut HindIII+blunt; after cut Apal, ligate with CNOT10 orf 496 nt HpaI/ApaI, cut out 5'UTR Hsp70 SacI/NdeI replace with 5'UTR CNOT10	cz4491, 4492, cz4710, cz4716
2301	CNOT10 stem loop TB927.10.8720 Puro: phd2300 cut HindIII/BamHI + ligate CNOT10 RNAi stem loop HindIII/BgIII	cz4688, 4689
2268	C-terminal myc-tagged CAF1: ORF cut HindIII/BamHI	cz4705, 4706
2264	CNOT10 KO BLA: 5'UTR XbaI/HindIII, 3'UTR XbaI/NotI	cz4032, 4033, 4034, 4035
2265	CNOT10 KO Hygro: 2264 Bla replaced with Hygro HindIII/XbaI	cz 4131, 4132
2275	N-terminal V5 in situ CAF40 Tb927.4.410 : 5'UTR NotI/XbaI, ORF XbaI/ApaI	cz4713, 4714, 4723, 4724
2342	λN-CAF1 C-terminal myc tag: ORF ApaI/XbaI	cz4422, 4660
2297	GFP + 6x B-Box:HindIII/ NheI	cz4327, 4328, 4331, 4332
2338	pGADT7 + Not1 C-terminal fragment NdeI/BamHI	cz4428, 4172
2339	pGADT7 + Not1 N-terminal fragment EcoRI/BamHI	cz4426, 4427
2340	pGBK7 + Not1 C-terminal fragment NdeI/BamHI	cz4428, 4172
2341	pGBK7 + Not1 N-terminal fragment EcoRI/BamHI	cz4426, 4427
2248	pGBK7 + CAF1 EcoRI/BamHI	cz4077, 4078
2249	pGBK7 + CNOT10 EcoRI/BamHI	cz4079, 4080
2257	pGBK7 + NOT5 NdeI/BamHI	cz4081, 4082
2251	pGADT7 + CAF1 EcoRI/BamHI	cz4077, 4078
2253	pGADT7 + CNOT10 EcoRI/BamHI	cz4079, 4080
2256	pGADT7 + NOT5 NdeI/BamHI	cz4081, 4082

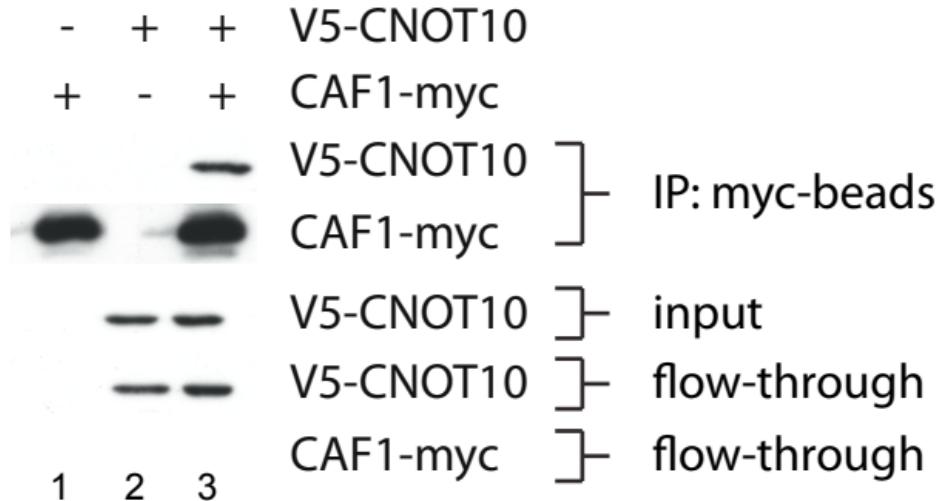
### 3B: Oligonucleotides

CZ oligos		
3919	gaga GCGGCCGC TCA CTG AAA GGA GTT TAA GCG	NotI
3920	gaga TCTAGA TTT CCC GTT TCC CGT TTC C	XbaI
3921	gaga CTCGAG ATG ATG CAG TAT GGT GGC G	XhoI
3922	gaga GGGCCC CGG AAG TTG AAC TGC CAC	Apal
4032	gaga CTCGAG AGA GAA TTC AGG AGA ATA ACT GG	XhoI
4033	gaga AAGCTT TTC TAC GTC CCT TCT GTC TC	HindIII
4034	gaga TCTAGA AGT TGT TTA TGT GGG GTC CTT C	XbaI
4035	gaga GCGGCCGC TTG ATC ATT CCT CCG TTG CTC	NotI
4131	GTCaaagctATGAAAAAGCCTGAACTCACCG	HindIII
4132	GAGtctagaTCTATTCCCTTGCCCTCGGAC	XbaI
4172	TATGGATCCCTTCTTGCCCTGAGTTGCG	BamHI
4276	AAGGGCCCATGGTGAGCAAGGGC	Apal
4277	ATCTCGAGGCCGTAACGCTTGACA	XhoI
4278	ATTCTAGACCGGGCTGCACG	XbaI
4279	TCGCTAGCTTACTTGTACAGCTCGTCCATG	NheI
4327	AAGGATCCTCCCTAACGCTTAACTAACTAAGTCA	BamHI
4328	AGGAGCTCCCTAGAGATAATATCCTCGA	SacI
4331	ATT CTA GAC CGG GCT GCA CG	XbaI
4332	TCGCT AGCT TACTTGTACAGCTCGTCCATG	NheI
4422	ATAGGGCCCATGCAGTATGGTGGCG	Apal
4491	actg gagctc ggcattcctgcatttc	SacI
4492	actg catatg TTC TAC GTC CCT TCT GTC	NdeI
4660	ATACTCGAGGCTATGACCCTTACCGC	XhoI
4688	GAGAagatctGCATGCTCAGCACAACCTAGCCATCG	BglII,SphI
4689	CGGAgaattcGTCGACCCATCAGTGGCATGTGGTAG	EcoRI,Sall
4705	GAGAAAGCTTATGATGCAGTATGGTGGCG	HindIII
4706	GAGAGGATCCGCTATGACCCTTACCGC	BamHI
4710	gagaGTTAACATGACGGATGATGTCTTGAG	HpaI
4713	ACGT CTCGAG ATGCACCAAACCCAAGCGTATT	NotI
4714	ACGT GGGCCC CCTTCATTATCCCACGGAG	XbaI
4715	ACGT CTCGAGA TGACGGATGATGTCTTGAGAC	XhoI
4716	ACGT GGGCCC CATTGAGCTTCTCGGAAAGAAC	Apal
4716	ACGT GGGCCC CATTGAGCTTCTCGGAAAGAAC	Apal
4723	ACGT GCGGCCGC GTTGTCCCCATCACAGAG	XhoI
4724	ACGT TCTAGA GGTACGAAAATCCTACAAA	Apal
4725	ACGT GCGGCCGC GCGCCATTCTGCTTCTTC	NotI
4726	ACGT TCTAGA TTCTACGTCCCTCTGTCTCA	XbaI

#### siRNA

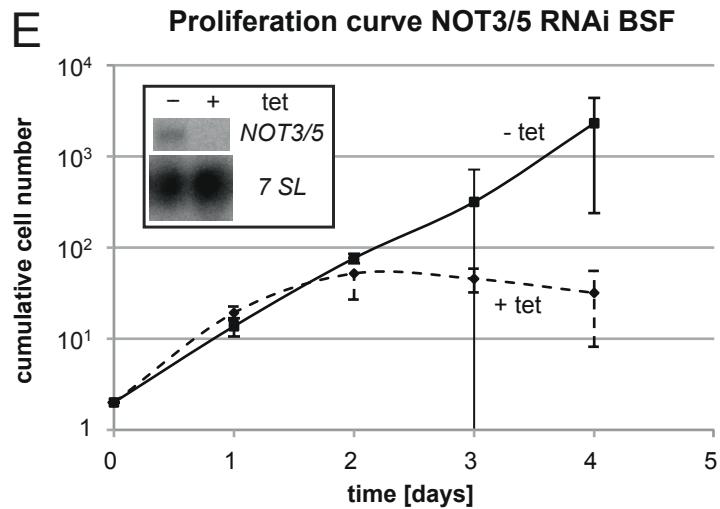
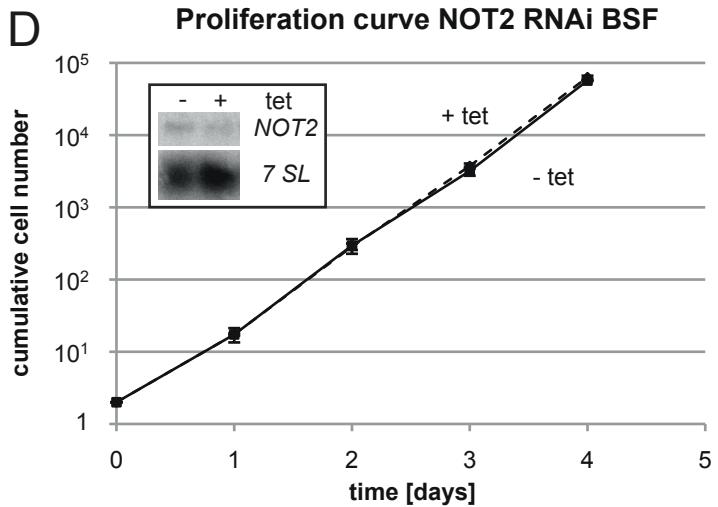
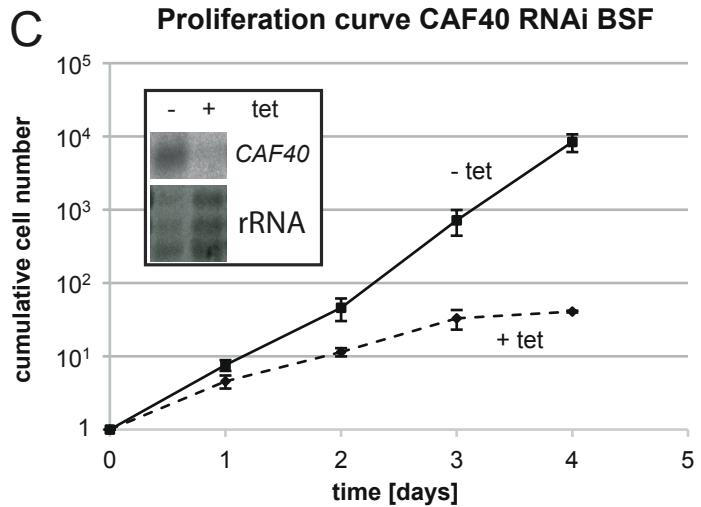
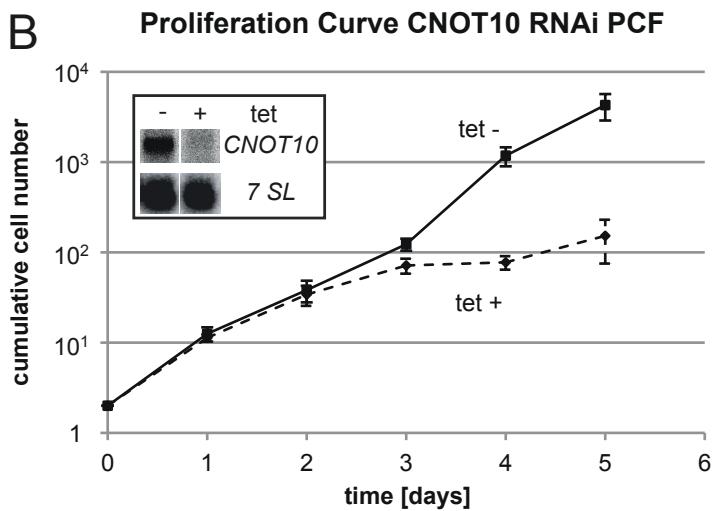
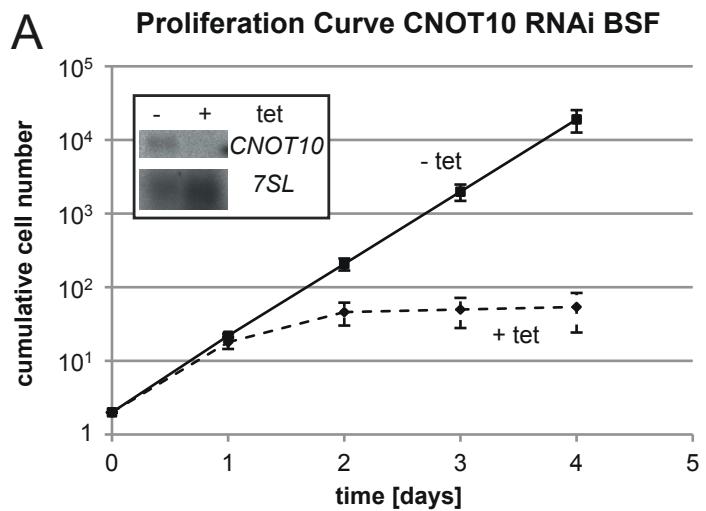
C2/S014 (control) 5'-GGUCCGGCUCCCCAAUUGdTdT-3';  
 S021 (CNOT1) 5'-GGACUUGUUUGAAGAAUAddTdT-3';  
 S059 (CNOT10) 5'-CAGCGAAAGCAGTGAAACTdTdT-3'.

### Supplementary Figure S1: Co-IP of CAF1-myc and V5-CNOT10



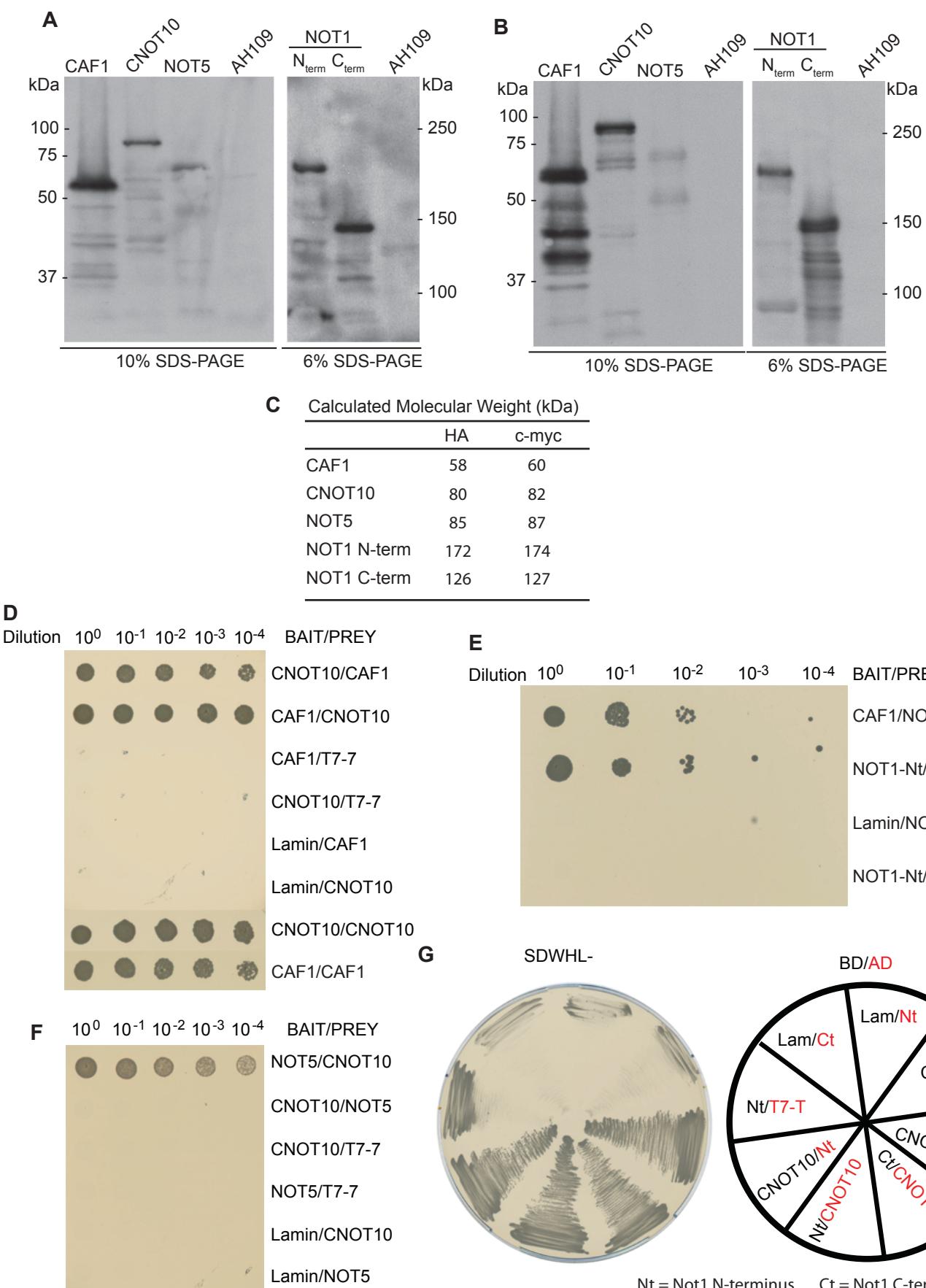
Using anti-myc beads, TbCAF1-myc was pulled down from extracts of trypanosomes expressing TbCAF1-myc alone,(lane 1) V5-TbCNOT10 alone (lane 2) or both tagged proteins (lane3). A Western Blot was probed to detect the myc and V5 tags. The top two panels show the precipitated V5-TbCNOT10 or TbCAF1-myc from the three different cell lines. The next panel below is 1% of the input extract, and the two bottom panels are the unbound fractions.

## Supplementary Figure S2



Trypanosomes with RNAi targeting different NOT complex subunits were grown with or without 100 ng/ml tetracycline to induce RNAi. Cells were diluted to  $2 \times 10^5$ /ml as necessary to avoid densities above  $4 \times 10^6$ /ml. Cumulative growth curves are shown. BSF = bloodstream form, PCF = procyclic form.

### Supplementary Figure S3



Yeast two-hybrid analysis of the trypanosome NOT complex. (A, B) Expression of trypanosome NOT complex subunits in yeast: Extracts of yeast AH109 expressing the indicated proteins were subjected to immunoblotting analysis using (A) anti-Myc (1:2000 dilution, Santa Cruz) or (B) anti-HA (1:2000 dilution, Roche) antibodies. (C) The expected sizes of the fusion proteins. (D,E,F) Growth of transformants on SDWHAL- media (lacking tryptophan, histidine, adenine, and leucine) indicates protein-protein interaction. From left to right, 10-fold dilutions of the initial cell suspension were plated into synthetic selective media. Yeast cells co-transformed with each control plasmid failed to grow on restrictive media. Photos were taken after 4 days of incubation at 30 °C. The positive clones were confirmed by β-galactosidase colony filter-lift assay (not shown). (G) Growth of CNOT10 interactors on less stringent medium, SDWHL- (lacking tryptophan, histidine, and leucine). These interactions were not seen under the more stringent conditions.